COMPARATIVE STUDY OF ER STATUS IN PREMENOPAUSAL AND POSTMENOPAUSAL WOMEN WITH CARCINOMA BREAST

DISSERTATION SUBMITTED FOR

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CHENNAI

CERTIFICATE

This is to certify that the dissertation entitled "**Comparative study of ER status in premenopausal and postmenopausal women with Carcinoma Breast**" is the bonafide work of **Dr.V.GOPISRI** in partial fulfilment of the university regulations of the Tamilnadu Dr. M.G.R. Medical University, Chennai, for M.S. (Branch I) General Surgery examination to be held in April 2013.

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DECLARATION

I , **Dr.V.GOPISRI**, hereby declare that, I carried out this work on **"Comparative Study of Estrogen Receptor Status in Premenopausal and Postmenopausal women with Carcinoma Breast"** at the Department of Surgery, Government Rajaji Hospital, Madurai ,under the guidance of **Prof.Dr. Nasheer Ahamed Syed, M.S.,** Professor of Surgery, during the period of September 2010 to August 2012. I also declare that this bonafide work has not been submitted in part or full by me or any others for any award , degree or diploma to any other University or Board either in India or Abroad.

This is submitted to the Tamilnadu Dr.M.G.R.Medical University, Chennai in partial fulfilment of the rules and regulations for the M.S. Degree Examination in General Surgery (Branch I) to be held in April 2013.

Place : Madurai

Date : **(Dr.V.Gopisri)**

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CONTENTS

INTRODUCTION

Breast cancer, which is the most common malignancy in women, was found to have association with estrogen The discovery of the estrogen receptor produced vast changes in the management of cancer breast .It is an important prognostic marker.

On treating patients with carcinoma of the breast we are often confronted with the dilemma of how to identify those patients who are most likely to benefit from hormonal treatment. Estrogen receptor rich tumors respond to hormone therapy more frequently than do estrogen receptor poor tumors. Estrogen receptor status is the most important indicator in predicting the response of advanced breast cancer to hormonal manipulation and possibly chemotherapy.

AIM AND OBJECTIVES

The aim of this work is:

1. To study the pattern of Estrogen Receptor status in female patients with breast cancer.
2. To find out eventual correlation between Estrogen Receptor status and menstrual status.
3. To predict the value of Estrogen Receptor status in choosing patients for adjuvant hormonal treatment.

REVIEW OF LITERATURE

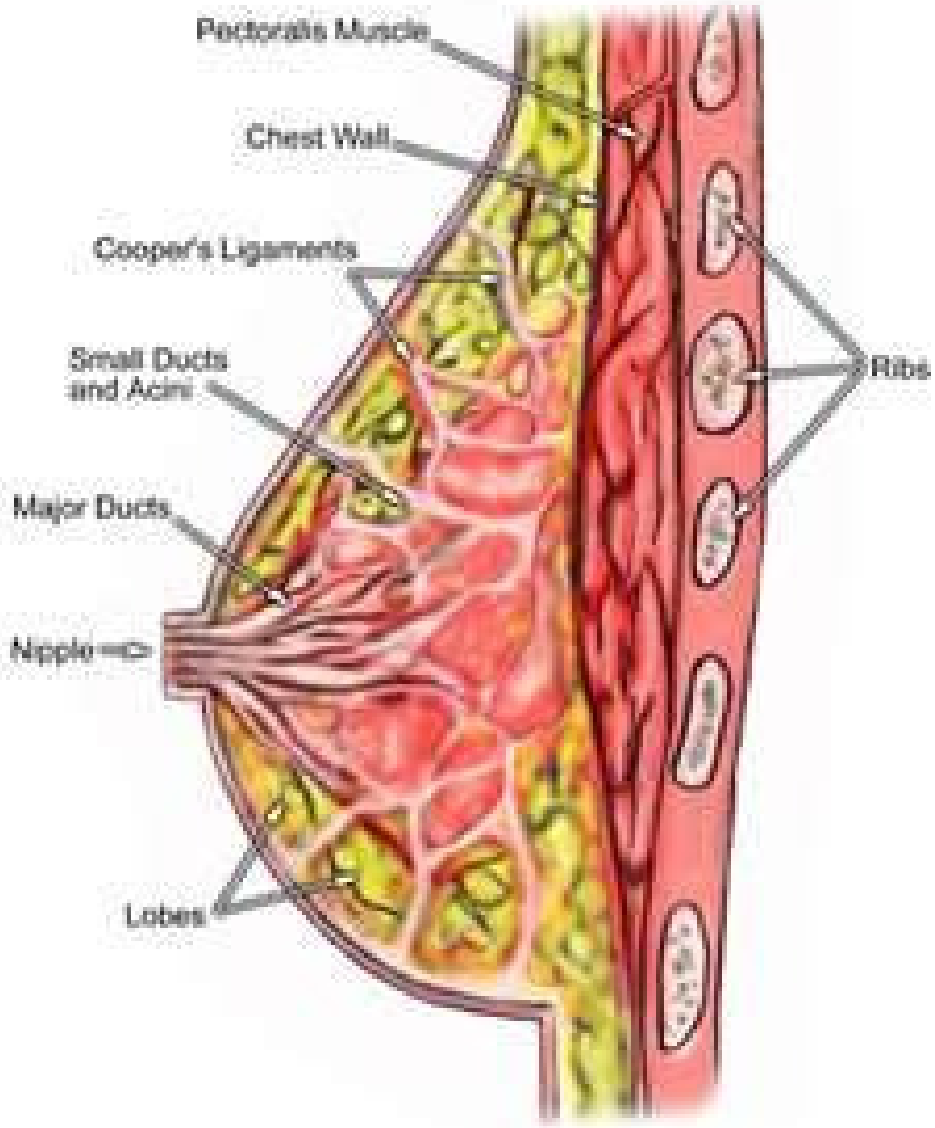
The levels of estrogen receptors had been studied in many cases of primary carcinoma of the breast and it was found that percentage of patients with estrogen receptor positive carcinomas was higher in postmenopausal group.

Relationship of age and menopausal status to estrogen receptor content in primary carcinoma of the breast was studied from the departments of medicine, pathology, surgery and biochemistry, Duke University Medical Center, Durban, North Carolina and was published in Annals of Surgery, February 1983, Vol 197.

The cytosolic estrogen receptor (CER) content of 1037 primary breast carcinomas was evaluated by sucrose density gradient analysis. Tumor specimens from premenopausal patients had significantly lower level of cytosolic estrogen receptor compared with carcinomas from postmenopausal patients.

The initial report by Jensen et al. said that the measurement of Cytosolic Estrogen Receptor content in metastatic breast carcinoma tissue was useful in predicting response to endocrine therapy. This determination has been widely used to select therapy. It has also been reported that patients with primary tumors that were CER rich experienced improved survival compared with patients whose carcinomas are CER poor. Compared with premenopausal patients, postmenopausal patients have quantitatively higher levels of CER and a greater proportion of their tumors are CER positive.

SURGICAL ANATOMY OF BREAST



ROLE OF ESTROGEN IN DEVELOPMENT OF BREAST

The major hormonal influence on the breast is estrogen. The initial immaturity of the hypothalamic–pituitary axis results in anovulatory cycles for the first 1–2 years after menses begin, subjecting the breast to the effects of unopposed estrogen. It is during this period of unopposed estrogen stimulation, considered an "Estrogen Window" that the ductal growth phase occurs.

A potent mammogen, estrogen primarily stimulates ductal growth but also increases fat deposition and contributes to later phases of development. Impaired ductal growth has been demonstrated in both people lacking the functional gene for the estrogen receptor (ER) and people treated with tamoxifen, an ER modulator. The estrogen receptor ER α is thought to be the key mediator of estrogen effects and in humans has only been documented in luminal epithelium.

EPIDEMIOLOGY OF BREAST CANCER

The incidence rates of breast cancer increased in most countries through the 1990s. Since the estimates for 1990, there was an overall increase in incidence rates of approximately 0.5% annually.

Recent data from the SEER program reveal declines in breast cancer incidence over the past decade and this is widely attributed to decreased use of hormone replacement therapy.

Breast cancer burden has well-defined variations by geography, regional lifestyle and racial or ethnic background. In general, both breast cancer incidence and mortality are relatively lower among the female populations of Asia and Africa, relatively underdeveloped nations and nations that have not adopted the westernized reproductive and dietary patterns. In contrast, European and North American women and women from heavily industrialized or westernized countries have a substantially higher breast cancer burden. These international patterns are mirrored in breast cancer incidence and mortality rates.

Although often related the factors that influence breast cancer incidence may differ form those that affect mortality. Incidence rates are lower among populations that are heavily weighted with women who begin childbearing at young ages and who have multiple full-term pregnancies followed by prolonged lactation.

Breast cancer mortality rates should be lower in populations that have a lower incidence, but the mortality burden will simultaneously be adversely affected by the absence of effective screening programs for early detection and diminished access to multidisciplinary cancer treatment programs. These features are likely to account for much of the disproportionate mortality risks that are seen in underdeveloped nations.

TRADITIONAL RISK FACTORS FOR BREAST CANCER

RISK FACTOR	COMPARISON CATEGORY	RISK CATEGORY
Age at menarche	16 years	Younger than 12 years
Age at menopause	45 – 54 years	After 55 years
Age when first child born alive	Before 20 years	Nulliparous or older than 30 years
Benign breast disease	No biopsy or fine needle aspiration	Any benign disease Proliferative disease Atypical hyperplasia
Family history of breast cancer	No first degree relative affected	Mother affected Two first degree relatives affected

NEWER EPIDEMIOLOGIC RISK FACTORS FOR BREAST CANCER

CHARACTERISTIC	MENOPAUSAL STATUS	COMPARISON CATEGORY	RISK CATEGORY
Circulating estradiol	premenopausal	Lowest quartile	Highest quartile
	Postmenopausal	Lowest quartile	Highest quartile
Circulating estrone	premenopausal	Lowest quartile	Highest quartile
	Postmenopausal	Lowest quartile	Highest quartile

NEWER EPIDEMIOLOGIC RISK FACTORS FOR BREAST CANCER

CHARACTERISTIC	MENOPAUSAL STATUS	COMPARISON CATEGORY	RISK CATEGORY
Genetic factors BRCA 1 mutation	Both	No mutation	Mutation present in gene
BRCA 2 mutation	Both	No mutation	Mutation present in gene
Hormonal factors Oral contraceptive use	Both	Never users	Current users
	Both	Never users	>10 years since last use
Hormone therapy use	Post menopausal	Non users with an intact uterus	Estrogen + progestin users
		Non users with a hysterectomy	Estrogen users

REPRODUCTIVE FACTORS

CHARACTERISTIC	MENOPAUSAL STATUS	COMPARISON GROUP	REFERENCE GROUP
Parity	Both	2 live birth	Nulliparous
Breast feeding	Premenopausal	Ever breastfed	Never breastfed
	Postmenopausal	Ever breastfed	Never breastfed

BEHAVIOURAL FACTORS

CHARACTERISTIC	MENOPAUSAL STATUS	COMPARISON CATEGORY	REFERENCE CATEGORY
BMI	Postmenopausal	<21 Kg per m2	>33 Kg per m2
	Premenopausal	<21 Kg per m2	>33 Kg per m2
Height	Postmenopausal	<1.60 m	>1.75 m
	Premenopausal	<1.60 m	>1.75 m
Weight	Postmenopausal	<60 Kg	>80 Kg
	Premenopausal	<60 Kg	>80 Kg
Alcohol use	Both	Never drunkers	>12 g per day
Smoking	Both	Never smokers	Smoked >40 years

SIGNS AND SYMPTOMS OF BREAST CANCER

1. Mass or swelling in the breast
2. Nipple ulceration
3. Nipple retraction
4. Peaud orange
5. Ulceration or fungation of breast

DIAGNOSTIC STUDIES FOR BREAST CANCER PATIENTS

CANCER STAGE

	0	**I**	**II**	**III**	**IV**
History & physical examination	X	X	X	X	X
Complete blood count		X	X	X	X
Liver function test		X	X	X	X
Chest radiograph		X	X	X	X
Hormone receptor status		X	X	X	X
HER- 2/neu expression		X	X	X	X
Bone scan			X	X	X
CECT abdomen or ultrasound			X	X	X

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MANAGEMENT OF CA BREAST

Surgical treatment

- Total Mastectomy
- Breast Conservation Therapy

Management of axilla

- SLN dissection
- Axillary node dissection

Chemotherapy

- Adjuvant
- Neoadjuvant

Endocrine therapy

Radiation therapy

Immuno therapy

HISTORY OF HORMONAL THERAPY AND ESTROGEN RECEPTORS

Hormonal treatment for breast cancer was considered even before the beginning of the twentieth century. In 1889, Albert Schinzinger of Freiburg , Germany , proposed oophorectomy before mastectomy to knock off estrogen in menstruating women .

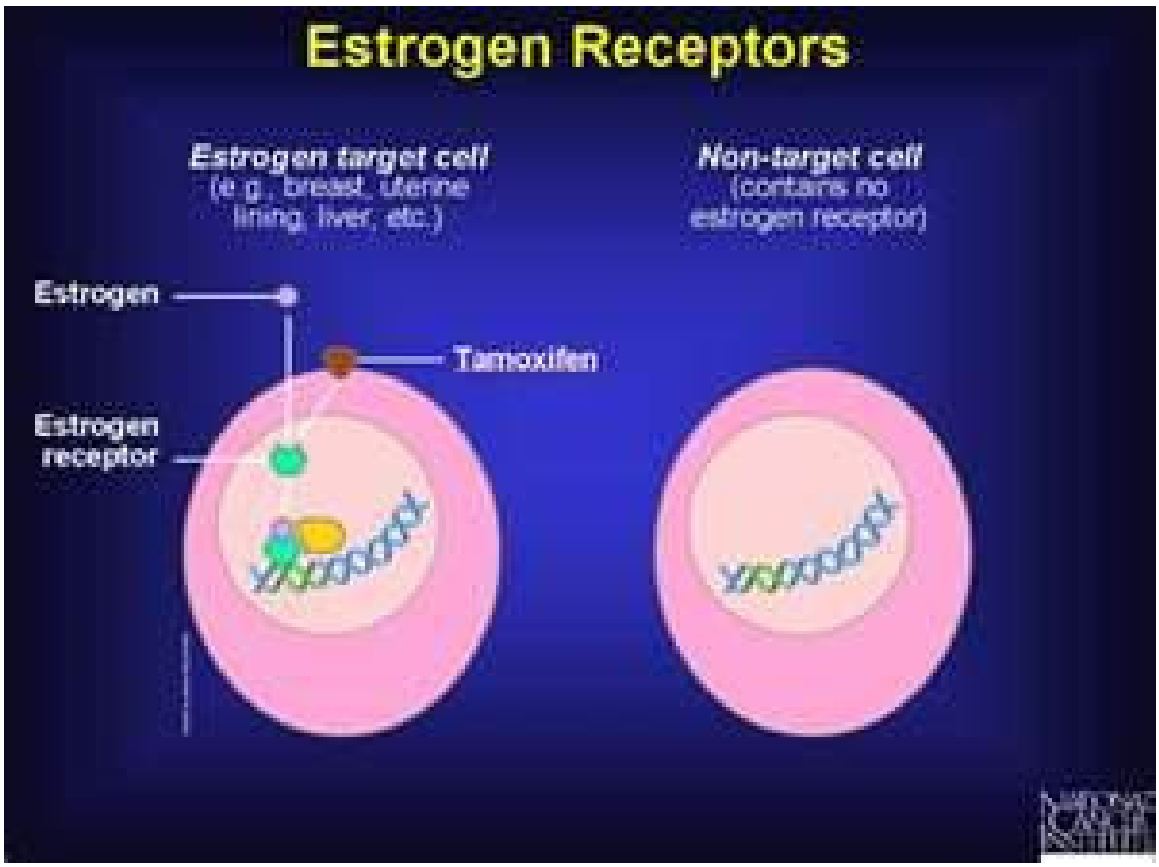
In 1973, W.Mc Guire demonstrated estrogen receptors in human breast tumors. In 1975, K. Horowitz identified progesterone receptors in hormone – dependent breast cancer .Since the 1980s, tamoxifen and other selective estrogen receptor modulators (SERMs) have been used for the treatment and prevention of breast cancer.

HISTORICAL BACKGROUND OF HORMONAL THERAPY

In the mid 1980s Dr.George T.Beatson presented a paper to the Edinburgh Medico–Chirurgical society stating that oophorectomy in rabbits resulted in loss of lactation . This led to the hypothesis that lactation is controlled by the ovaries and that removal of the ovaries could potentially benefit patients with breast cancer.

Based on his hypothesis, Dr.Beatson performed an oophorectomy on June 15, 1895 on a premenopausal patient with advanced, unresectable breast cancer with soft tissue recurrence . The patient showed clinical improvement, had a complete remission and survived four years after the surgery. This finding was the first clinical evidence that breast cancer may be dependent on ovarian function .

The benefit of oophorectomy in some breast cancer patients was confirmed by Dr.Stanley N.Boyd with a case report and a subsequent series of 46 premenopausal women with advanced breast cancer.


Estrogen Receptors
Estrogen target cell
(e.g., breast, uterine lining, liver, etc.)
Non-target cell
(contains no estrogen receptor)
Estrogen
Tamoxifen
Estrogen receptor

ADJUVANT ENDOCRINE THERAPY

Tamoxifen is the historic standard for adjuvant endocrine therapy for breast cancer. Tamoxifen administered for five years results in a 41% reduction in the annual rate of breast cancer recurrence and 34% reduction in annual death rate for women with estrogen receptor positive breast cancer .

Shorter durations of tamoxifen therapy are also beneficial , but appear to have less impact than 5 year treatment duration. The optional duration of tamoxifen therapy appears to be 5 years ; extending tamoxifen therapy beyond 5 years in patients with no evidence of tumor recurrence has not led to further improvement in disease free or overall survival. Consistent with its activity as a SERM, tamoxifen is not effective in preventing recurrence of hormone receptor negative breast cancer.

ENDOCRINE THERAPY IN DCIS

Two prospective randomised trials have evaluated the effect of tamoxifen on outcome in patients with DCIS treated with breast conserving therapy. In the NSABP B – 24 trial, 1804 women with DCIS were randomly assigned to breast conserving surgery and radiation therapy followed by either tamoxifen at 20 mg / day or a placebo for 5 years. At 7 years of follow up, women who received tamoxifen had fewer breast cancer events than did placebo group (10% vs. 16.9%) . Among those who received tamoxifen, the rate of ipsilateral invasive breast cancer was 2.6% at 7 years compared to 5.3% in the control group. Tamoxifen also decreased the 7-year cumulative incidence of contralateral breast neoplasms (invasive and non invasive) to 2.3% compared with 4.9% in the control group.

A subgroup analysis, based on estrogen receptor status, indicated that women with estrogen receptor positive DCIS who received tamoxifen had a 59% reduction in their relative risk of breast cancer events when compared with those who received the placebo .Among patients with estrogen receptor negative DCIS there was no added benefit from tamoxifen.

The decision of whether to use adjuvant tamoxifen for patients with DCIS should be made on an individual basis. The use of tamoxifen has been associated with vasomotor symptoms, deep vein thrombosis, pulmonary embolism and increased cataract formation. The risk of endometrial cancer is increased two to seven times among patients who receive the drug. Tamoxifen is also associated with increased risk of stroke and benign ovarian cysts. Therefore, the effects of tamoxifen to reduce ipsilateral breast tumors and to prevent contralateral breast disease should be weighed against the risk of tamoxifen use in each patient. In addition, tamoxifen should be reserved for patients with estrogen receptor positive tumors.

Aromatase inhibitors have been shown to be beneficial in the adjuvant treatment of breast cancer in postmenopausal women with estrogen receptor positive disease. These agents have fewer cardiovascular side effects than tamoxifen and may be beneficial in the adjuvant treatment of patients with DCIS following breast conserving surgery.

ENDOCRINE THERAPY IN LCIS

For patients with a diagnosis of LCIS, one of the treatment options is chemoprevention with tamoxifen . In the NSABP P-1 breast cancer prevention trial , Fisher et al. (1998) observed a 56% decrease in the incidence of invasive breast cancers in the subset of women with LCIS who received tamoxifen as compared with women with LCIS who underwent observation alone. The annual hazard rate of invasive cancer was 5.69 per 1000 women who received tamoxifen compared with 12.99 per 1000 women who did not.

In the NSABP P-2 trial, postmenopausal women with LCIS were eligible to be randomised between tamoxifen and raloxifene. Vogel et al.(2006) reported that the two agents offered an equivalent risk reduction for invasive breast cancer (incidence 4.30 per 1000 vs. 4.41 per 1000, for tamoxifen and raoxifen, respectively). Patients receiving raloxifen had a lower risk of thromboembolic events and cataracts.

Vogel et al. concluded that depending on an individual's personal risk factors, both raloxifen and tamoxifen are valuable for breast cancer risk reduction. Raloxifen may be particularly beneficial to a postmenopausal women with an intact uterus who also faces a risk of osteoporosis.

ENDOCRINE THERAPY IN INVASIVE BREAST CANCER

Endocrine therapy has been associated with significant reductions in

1. Risk of local recurrence
2. Distant metastasis
3. Contralateral breast cancer

Tamoxifen therapy

Tamoxifen which was originally recommended for the treatment of postmenopausal women with ER – positive breast cancer , is now indicated for a much broader range of patients. Tamoxifen therapy is considered standard of care for premenopausal women with tumors expressing estrogen receptor , regardless of age or nodal status.

Tamoxifen therapy is generally well tolerated ; treatment limiting adverse effects develop in less than 5% of patients. In addition to its antitumor properties, tamoxifen increases bone density and reduces serum cholesterol levels. However, tamoxifen also increases the incidence of endometrial cancer and thrombo embolic events.

A meta – analysis of five randomized clinical trials showed that patients who were treated with tamoxifen for 3-5 years had a greater reduction in recurrence than did patients treated for 1 to 2 years . Data from NSABP B-14 indicated that 10 years of tamoxifen offer no survival advantage over 5 years.

Most recently in the EBCTCG analysis, in women with ER-positive tumors were randomised to receive 5 years of adjuvant tamoxifen, there was a 41 % proportional risk reduction of recurrence and there was a 34% proportional risk reduction of mortality compared with those who did not receive tamoxifen. Currently 5 years of adjuvant tamoxifen is considered the standard therapy for premenopausal women.

Current ASCO recommendations for postmenopausal women include an aromatase inhibitor as a component of adjuvant endocrine therapy. The aromatase inhibitors anastrazole, letrozole and exemestane work by inhibiting the aromatase enzyme that catalyses the conversion of adrenal corticosteroids to estrogens and therefore decreases the conversion of precursor hormones to estrogen in adipose tissue .

In the ATAC (Arimidex, Tamoxifen Alone or in combination) trial, the efficacy and side effect profiles of anastrazole and tamoxifen were compared in postmenopausal women. For postmenopausal patients with early stage breast cancer, anastrazole resulted in a higher DFS rate (86.9% vs. 84.5%) than tamoxifen ,

longer time to recurrence and lower incidence of contralateral breast cancer.

The results also demonstrated that the incidence of endometrial cancer, vaginal bleeding and discharge, cerebrovascular events, venous thromboembolic events and hot flushes occurred significantly less frequently witn anastrazole; whereas musculoskeletal disorders and fractures occurred less frequently with tamoxifen. As a result of ATAC trial, anastrazole is now the preferred hormone therapy for postmenopausal patients with receptor positive breast cancer.

ENDOCRINE THERAPY IN ER POSITIVE CASES

The estrogen receptor is present in about in 80% of DCIS lesions and is more frequent in non comedo than comedo DCIS. Endocrine therapy has two potential benefits in women with breast cancer : a reduction in local recurrence after breast conserving therapy and the prevention of the development of new primary breast cancers in the contralateral breast .

In women with ER – positive breast cancer, tamoxifen reduced the risk of any breast cancer event by 59 % . In women with ER – negative tumors, no significant benefit was seen.

Overview of Adjuvant Treatment Approaches in Breast Cancer

	Hormone Receptor Status POSITIVE	Hormone Receptor Status NEGATIVE
Tumor HER Status	POSITIVE	NEGATIVE
HER-2 negative / normal	Endocrine therapy +Chemotherapy	Chemotherapy
HER-2 positive / overexpressed	Endocrine therapy + Chemotherapy + Trastuzumab	Chemotherapy + Trastuzumab

ENDOCRINE THERAPIES USED FOR METASTATIC BREAST CANCER

Aromatase Inhibitors

Non-steroidal

Anastrazole	1 mg orally daily	Hot flushes , arthralgias , myalgias, headaches , diarrhoea , mild nausea
Letrozole	2.5 mg orally daily	

Steroidal

Exemestane	25 mg orally daily

Antiestrogens

SERMs

Tamoxifen	20 mg orally daily	Hot flushes, vaginal discharge, mild nausea, thromboembolism , endometrial Ca
Toremifene	60 mg orally daily	

SERDs

Fulvestrant	250 mg i.m. every 28 days	Hot flushes , injection site reactions , thromboembolism

ANDROGENS

Fluoxymestrone	10 mg orally twice a day

ESTROGENS

Diethylstilbestrol	5 mg orally 3 times a day
Ethinylestradiol	1 mg orally 3 times a day
Conjugated estrogens	2.5 mg orally 3 times a day

LHRH analogs

Goserelin	3.6 mg s.c. every 28 days
Leuprolide	3.75 mg i.m. every 28 days
Triptorelin	3.75 mg i.m. every 28 days

PROGESTINS

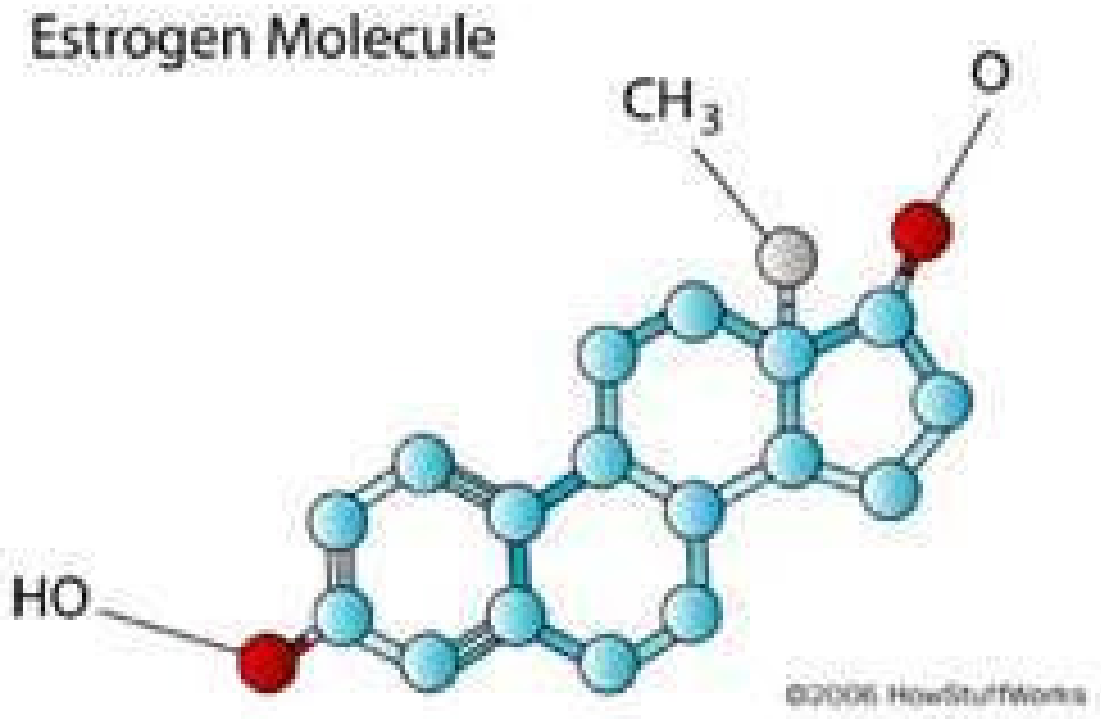
Megestrol acetate	40 mg orally four times a day
Medroxyprogesterone	400-1000mg i.m. every week

ESTROGEN RECEPTORS IN BREAST CANCER

Estrogen is a steroid hormone, synthesized by ovaries and other tissues, that is essential for normal mammary development as well as lactation. Estrogen mediates its activities in target tissues by activating estrogen receptors, which exhibit transcriptional (genomic) and membrane localised (nongenomic) signalling activities. The transcriptional activity in targeted tissue occurs in co-operation with coactivator proteins that interact directly or indirectly with estrogen responsive genes facilitating mammary tissue proliferation.

The physiologic functions of estrogen signalling pathways are co-opted in breast cancer to promote cancer progression. There are two major receptors for estrogen of the nuclear hormone receptor family, ERα and ERβ. The majority of the breast cancers i.e., 70% expresses the estrogen receptor.

The estrogens are a family of hormones synthesized in a variety of tissues. 17 - estradiol is the primary estrogen of ovarian origin . In pregnancy, relatively more estriol is produced and this comes from placenta.


Estrogen Molecule
CH3
O
HO
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Estrogens are formed by the aromatization of androgens in a complex process that involves three hydroxylation steps ; each of which requires 02 and NADPH .The aromatase enzyme complex is thought to include a P450 monooxygenase. Estradiol is formed if the substrate of this enzyme complex is testosterone, whereas estrone results from the aromatization of androstenedione.

Theca cells are the source of androstenedione and testosterone; these are converted by the aromatase enzyme in granulosa cells to estrone and estradiol, respectively .

The whole concentration of estradiol in plasma during menstrual cycle comes from the ovary and there are two peaks of secretion : one just before ovulation and one during the midluteal phase .

The estradiol secretion rate is :

Early follicular phase	-	36 microgm / day
Just before ovulation	-	380 microgm / day
During midluteal phase	-	250 microgm / day

After menopause, estrogen secretion declines to low levels. The human ovaries become unresponsive to gonadotropins with advancing age and their function declines, so that sexual cycles disappear (menopause). This unresponsiveness is associated with and probably caused by a decline in the number of primordial follicles. The ovaries no longer secrete progesterone and 17-estradiol in appreciable quantities and estrogen is formed only in small amounts of aromatization of androstenedione in peripheral tissues.

Significant amounts of estrogens are produced by the peripheral aromatization of androgens. In females, adrenal androgens are important substrates since as much as 50% of the estradiol produced during pregnancy comes from the aromatization of androgens. Conversion of androstenedione to estrone is the major source of estrogens in postmenopausal women. Aromatase activity is present in adipose cells and also in liver, skin and other tissues.

In 1923, an ovarian hormone regulating mammary tissue, estrogen was discovered by Drs. Edgar Allen and Edward Doisy. Nearly 40 years passed, until Jensen and Jacobson synthesized

radioactive estradiol (E2). The observation of tissue specific localisation of E2 led to the hypothesis that there is a specific estrogen receptor.

In 1966, the estrogen receptor was purified and characterised, leading to the subsequent findings that breast tissue expresses ER and some breast cancers contain ER. Furthermore E2 was linked to cellular proliferation and differentiation in normal tissues .The concentration range under which hormone binding occurs varies depending on biologic conditions. E2 concentrations much lower than 10-12 M are capable of stimulating ER function.

ESTROGEN RECEPTORS

Like other steroids, estrogens combine with protein receptors in the nucleus and the complexes bind to DNA, promoting formation of mRNAs that in turn direct the formation of new proteins which modify cell function.

Two nuclear estrogen receptors have been cloned :

ER α - encoded by a gene on chromosome 6

ER β - encoded by a gene on chromosome 14.

Although there is overlap, the distribution of these receptors is different.

ER α - expression is moderate to high in :

Uterus, testis, breast, cervix , vagina, pituitary, kidney, epididymis and adrenal.

ER β - expression is high in :

Ovary, prostate, lung, bladder and vascular tissue.

Both ER α and ER β in :

Brain, CVS and bone.

It has been suggested that the regulation of ovarian function by the pituitary ovarian axis is primarily ER α mediated ; whereas the estrogens secreted into the ovarian follicles act primarily via ER βs.

STRUCTURE OF ESTROGEN RECEPTORS

The two forms of the ER , ER α and ERβ are encoded by different genes . ESR1 maps to chromosome 6q25.1 whereas ESR2 maps to 14q23.2. After translation , ERs are initially complexed with heat shock protein 90 (Hsp 90) ; they then dimerize when exposed to estrogen. Dimerization can also be facilitated by DNA binding

.

ER α (ESR1) and ER β (ESR 2) may form homodimers or heterodimers. The six domains of ER α and ER β are from N to C terminus. A/B containing transcription activating domain AF1 ; C containing the DNA binding domain ; D, which is an interdomain region ; E, which is a ligand binding domain containing transcription activating domain AF2 ; F , which contains the C terminal domain .

STRUCTURE OF THE ESTROGEN RECEPTOR

The A/B contains the activator function region AF1.

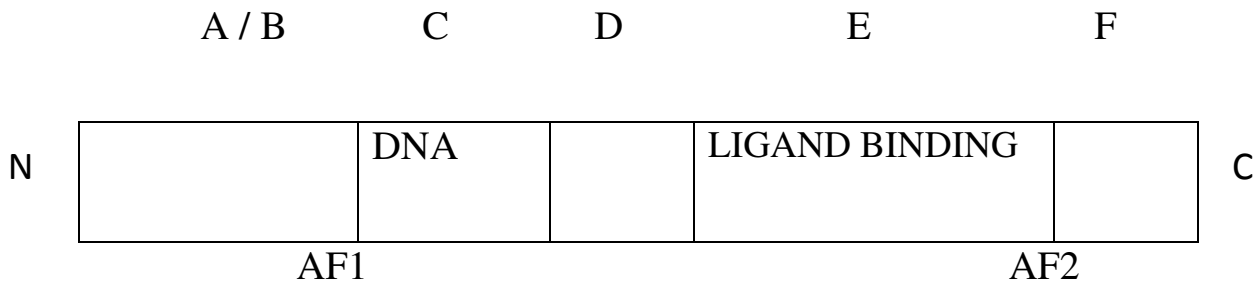
C domain contains – DNA binding domain .

D domain is a spacer.

E domain contains ligand binding function.

E domain also contains the activator region AF2.

F domain is a carboxy terminal domain.



Molecular Cloning and Characterization of The Estrogen Receptors

There are two ubiquitous ERs, ER α and ERβ. But the relative abundance of each receptor determines, in part the tissue specificityof their effector functions .The molecular era of estrogen biology began with the molecular cloning and sequencing of the ERα - c DNA and its gene (ESR1).

Both estrogen receptors exhibit E2 – dependent DNA binding and mediate transcriptional regulation through interaction with estrogen response elements (EREs) that may promote or inhibit transcription

ER α exhibits regulatory phosphorylation sites at Ser106, Ser118, Ser236 and Tyr537 among others .In addition to epidermal growth factor signalling, which can activate ER-mediated transcription through MAPkinase – mediated ER phosphorylation, the IGF pathway can also promote ER-mediated transcription .

EXPRESSION OF ESTROGEN RECEPTORS IN BREAST CANCER

About 70% of breast cancers express ER α detectable by immunohistochemistry. The expression of ER α in breast cancer is constitutive, in contrast to the normal mammary epithelium, where proliferating cells fail to exihibit ER expression and ER α expression is confined to a small proportion of non proliferating luminal cells .

It has been hypothesized that ER α could play a role in the etiology of breast cancer under conditions of prolonged exposure to estrogens such as those occurring with early menarche, late menopause, obesity or hormone replacement therapy. Prolonged exposure to E2 could lead to increased proliferation of mammary epithelial cells, increasing the risk of mutation events. Another hypothesis is that high E2 levels lead to increased metabolism of E2 to carcinogenic derivatives such as the reactive catechol 4-hydroxy-E2 which can damage DNA and lead to mutation .

ER α and immunohistochemical detection of ER α is predictive of response to hormonal therapy. ER α is cyclically regulated to the menstrual cycle in normal mammary tissue, but it is constitutively expressed in the most common subtypes of human breast cancer wherein it functions as a master regulator, promoting breast cancer growth and disease progression .

In contrast ER β exhibits an overlapping but distinct, tissue distribution of its pattern of expression in mammary tissue, cardiovascular tissue, the gastrointestinal tract and bone. The role of ER β in breast cancer is less well characterised.

On the basis of extensive clinical trial data in the 1970s and since then , ER α has been developed clinically as classifier of breast cancers that respond to hormonal therapy. ER α can be targeted with selective estrogen receptor modulators (SERMS) such as tamoxifen that compete with estrogen and alter the conformation of the ligand –binding of the receptor. SERMs such as tamoxifen are effective in

the metastatic and adjuvant settings and tamoxifen itself has also been successful in chemoprevention of breast cancer in women with elevated risk of breast cancer. Another approach for hormonal therapy is exemplified by the antiestrogen drug fulvestrant which promotes proteolytic degradation of ER .

STEROID HORMONE RECEPTOR IN CA BREAST

Hormones play an important role in the development and progression of breast cancer. Estrogens, estrogen metabolites and progesterones have been shown to have some effect. Breast cancer risk is related to estrogen exposure over time. In postmenopausal women, hormone replacement therapy increases the risk of breast cancer by 26%.

Patients with hormone receptor positive tumors survive two to three times longer after a diagnosis of metastatic disease than do patients with hormone receptor negative tumors. Patients with tumors negative for both estrogen receptors and progesterone receptors are not considered candidates for hormonal therapy. Tumors positive for estrogen or progesterone receptors have a higher response rate to endocrine therapy than tumors that do not express estrogen or progesterone receptors. Tumors positive for both receptors have a response rate of $> 50\%$; tumors negative for both receptors have a response rate $< 10\%$ and tumors positive for one receptor but not the other have an intermediate response rate of 33%.

The tumor hormone receptor status should be ascertained for both premenopausal and postmenopausal patients to identify patients who are most likely to benefit from endocrine therapy. Estrogen and progesterone receptor status can be measured in archived tissue using immunohistochemical techniques. Hormone receptor status also can be measured in specimens obtained with fine- needle aspiration or core needle biopsy and this can help in planning the treatment.

In a landmark breast cancer prevention study, tamoxifen exhibited efficacy as a preventive agent in patients with increased risk for breast cancer estimated by the Gail model , demonstrating proof of principle of chemoprevention in “at risk” patient population (National Surgical Adjuvant Breast and Bowel Project [NSABP] P-1).

Although ER is predictive of response to hormonal therapy rather than prognostic, a prognostic risk model for recurrence in patients with ER – positive breast cancer receiving adjuvant tamoxifen has been developed.

The utility of ER testing was subsequently established when investigators found that patients responding to tamoxifen exhibited higher levels of ER , leading to the further study and refinement of endocrine therapy for ER – positive breast cancer in clinical trials during the past 40 years .

Most notably researchers demonstrated that 5 years of tamoxifen was effective for prevention of breast cancer recurrence when added to adjuvant chemotherapy in node positive patients.

ESTROGEN RECEPTOR AND HORMONAL STATUS

The reason for the cytosolic estrogen receptor (CER) increasing with patient age may be related to a number of factors . Since postmenopausal women have lower circulating estrogen levels, the higher CER levels observed in tumors from these patients have been suggested to be the result of an increase in unoccupied cytosolic receptor rather than an increase in total cytosol receptor.

Saez et al. have postulated that the cyclic levels of serum progesterone in premenopausal patients limit CER synthesis .This later hypothesis is supported by the menstrual cycle variations of CER observed in normal human endometrium. In both the breast and endometrium, preluteal CER values were significantly higher than CER levels during the luteal phase when plasma progestin is high . Thus the higher CER levels observed in postmenopausal patients may be related to chronic unopposed estrogen stimulation to a decrease in the progesterone down-regulation of CER or a combination of these factors.

ER POSITIVITY IN POSTMENOPAUSAL WOMEN

Postmenopausal patients had significantly higher CER levels than premenopausal patients. The cumulative distribution of CER by menstrual status demonstrated a higher percentage of premenopausal patients with unmeasurable CER levels.

Examination of the CER values against age revealed steadily increasing CER values with age from third decade into the tenth decade. Tumors in patients < 35 years had quantitatively lower receptor values.

ER positivity increases in postmenopausal women. This may be seen as unusual because the ovaries may stop producing estrogen .In postmenopausal women, body fat still produces estrogen. But in this low estrogen environment , the breast cancer tries to expand the number of locks – the estrogen receptors – inside the tumor ; so that it can capture these very, very, small levels of estrogen . So estrogen receptor levels tend to be higher in postmenopausal women than in premenopausal women.

The integrity of receptor regulation of breast carcinoma growth is reflected in the tumor's biologic behaviour. The improved survival and response rate to hormonal therapy observed in elderly patients with breast carcinoma suggests that these patients would be more likely to have an intact receptor control mechanism, compared with younger patients.

SIGNIFICANCE OF ER STATUS

1. The frequency of ER positivity increase steadily with age.
2. The concentration and proportion of ER positivity were higher in postmenopausal than in premenopausal patients.
3. The level of ER positivity decreases with stage advancement.
4. Level of ER positivity decreases with increasing size of the primary tumor .
5. The more the concentration of ER, the little the chance to develop distant metastases .
6. ER assessment is essential for determining postoperative treatment modality .
7. ER positive patients receiving hormonal treatment have better prognosis .

IMMUNOHISTOCHEMISTRY

Immunohistochemistry otherwise called immunocytochemistry, is a method by which we identify specific antigens in tissues or cells . this is based antigen – antibody recognition ;One of the sensitive detection system is the enzymatic label (horseradish peroxidase) developed by Avrameas and colleagues, which ,in the presence of a suitable colorogenic substrate system, allowed visualization of the labelled antibody by orthodox light microscopy. Multiple– step detection techniques such as the peroxidase antiperoxidase (PAP), avidin–biotin conjugate (ABC) and biotin – streptavidin (B-SA) methods, together with amplification methods (such as tyramide) and the highly sensitive "polymer based" labelling systems.

The development of the hybridoma technique facilitated the development of IHC and the manufacture of abundant, highly specific monoclonal antibodies, many of which found early application in staining of tissues. Initial success in cryostat sections was eventually extended to routinely processed paraffin, celloidin, or other plastic – embedded tissue sections. The critical significance of

rendering the IHC technique suitable for routine paraffin sections was illustrated by Taylor and colleagues, who in 1974 showed that it was possible to demonstrate at least some antigens in routinely processed tissue. The collective appetites of pathologists worldwide, once whetted by these initial studies, led to serious attempts to improve further the ability to perform IHC staining on formalin – paraffin sections. Although great effort has been expended in the search for alternative fixatives (formalin fixatives) in order to preserve antigenicity without compromising preservation of morphologic features, no ideal fixatives have been found to date , as was pointed out by Larsson who said, “An ideal immuno cytochemical fixative applicable to all antigens may never be found”. In addition, preservation of morphologic features is not always comparable with formalin fixation.

Enzyme digestion was introduced by Huang and colleagues as a pretreatment to IHC staining to “unmask” some antigens that had been altered by formalin fixation. However, the enzyme digestion method, while widely applied , did not improve IHC staining of the majority of antigens, as reviewed by Leong and colleagues . Another

drawback of enzyme digestion was that it proved difficult to control the optimal digestion conditions for individual tissue sections when stained with different antibodies. These difficulties in standardization provided a powerful incentive for the development of a new technique, with that it should be more powerful, more widely applicable and easier to use than enzyme digestion. In addition, it should enhance immunohistochemical staining of routinely formalin fixed, paraffin embedded tissue sections in a reproducible and reliable manner.

The antigen retrieval technique, based on a series of biochemical studies by Fraenkel-Conrat and coworkers, was developed by Shi and associates in 1991 .In contrast to enzyme digestion, the AR technique is a simple method that involves heating routinely processed paraffin sections at high temperature (e.g., in a microwave oven) before IHC staining procedures. An alternative method that did not use heating was developed for celloidin-embedded tissues. The intensity of IHC staining was increased dramatically after AR pretreatment, as demonstrated by the original articles and more than 100 articles published

subsequently. Various modifications of AR technique have been described, the majority of which use different buffer solutions as the AR solution in place of metal salt solutions, which may have a potentially toxic effect. Worldwide applications of AR-IHC in pathology has validated the feasibility of AR-IHC and expanded its use in molecular morphology.

BASIC PRINCIPLES OF IMMUNOHISTOCHEMISTRY

In certain circumstances, the histochemical stains is proved to be of critical value in specific cell identification. More often, they served merely to highlight or emphasize cellular or histologic features that supported a particular interpretation without providing truly specific confirmation . Now-a –days there is possibility to use specific stains in the field of immunohistochemistry .

The aims of IHC are akin to those of histochemistry. Indeed, IHC builds on the foundations of histochemistry ; it does not replace histochemistry but rather serves as a valuable adjunct that greatly extends the variety of tissue components that can be demonstrated specifically within tissue sections or other cell preparations. . The basic critical principle of IHC, as with any other special staining method, is a sharp localization of target components in the cell and tissue, based on a satisfactory signal-to –noise ratio. Amplifying the signal while reducing nonspecific background staining (noise) is the

major strategy to achieve a satisfactory and practically useful result. After more than two decades, advances in IHC have provided a feasible approach to performing immunostaining on routinely processed tissues.

ANTIBODIES AS SPECIFIC STAINING REAGENTS

Evaluation of an antibody for use in IHC is based on sensitivity and specificity of the antigen-antibody reaction for IHC. Although the specificity of monoclonal antibody has been questioned regarding cross-reactivity with nontarget molecules, most commercially available monoclonal antibodies are highly reliable for IHC.

TISSUE FIXATION AND PROCESSING

Tissue preparation consists of fixation, subsequent dehydration and embedment in paraffin wax to provide a rigid matrix for sectioning .Tissues that are to be embedded in paraffin wax are first fixed in order to optimize preservation, a process that profoundly affects the morphologic and immunohistologic results. The ideal fixative for IHC studies should not only be readily available but should also be in widespread use to maximize the range and number of samples available for IHC studies .The fixative should preserve antigenic integrity and should limit extraction, diffusion or displacement of antigen during subsequent processing .

Common fixatives used in histopathology are divided into two groups: coagulant fixatives, such as ethanol and cross linking fixatives such as formaldehyde. Both types of fixatives can cause changes in the steric configuration of proteins, which may mask antigenic sites (epitopes) and adversely affect binding with antibody. It is well recognized that cross linking fixatives alter the IHC results for a significant number of antigens, whereas coagulant fixatives, especially ethanol, have been reported to produce lesser changes. In

most surgical pathology laboratories, the fixative used is 10% neutral buffer formalin (NBF) (a cross-linking fixative). Subsequent processing usually includes a period in 100% ethanol ; thus tissues are effectively double fixed in both formalin and ethanol . A long history of using formalin as a standard tissue fixative has revealed the following advantages :

1. There is good preservation of morphology for a variety of tissues, even after prolonged fixation.

2. Formalin is an economical chemical, much cheaper than most alternatives.

3. Formalin fixation can sterilise tissue specimen in a more reliable way than precipitating fixatives, particularly for viruses

4. Carbohydrate antigens are better preserved .

5. There is preservation of many antigens through cross-linking of protein in situ, thereby avoiding leaching out of proteins that may diffuse in water or alcohol.

Formalin may be regarded as a satisfactory fixative for both morphology and IHC provided that a simple and effective AR technique is available to recover those antigens that are diminished or modified.

HORMONE RECEPTORS IN IMMUNOHISTOCHEMISTRY

Estrogen receptors bind hormones that exert their effects in the nucleus. Nuclear immunostaining for estrogen receptor proteins can be demonstrated in normal breast acini, which serve as internal controls for the testing procedure. Nuclear staining in normal breast tissue is heterogenous and varies with the menstrual cycle.

Since the early 1990s, the immunohistochemical assay (ICA) determination of ER levels has replaced the dextran –coated charcoal (DCC) method .Some of the advantages of the ICA method include:

1. histologic documentation of the tumor tissue to be assayed .
2. appreciation of the heterogeneity of ERs in tumor nuclei .
3. rapid turnaround time.
4. lower cost.
5. ability to use minute quantities of tissue.

Some of the first ICA antibodies to be used would work well only on frozen tumor samples. The H222 antibody functioned well on frozen tissue , but subsequently studies on formalin – fixed,

paraffin embedded tissues stressed the importance of short (< 24 hr) formalin fixation times for optimal results .

The appearance of second generation ER antibodies (ER1D5 , DAKO, Carpinteria , CA) combined with newer antigen retrieval methods has rendered the DCC method obsolete .

Pertschuk and colleagues and Taylor and associates argued that using a percentage of nuclear staining of 10% as a minimum for a positive result was reproducible and correlated well clinically.

Barnes and colleagues used a triad of staining intensity, percent positive cells and degree of heterogeneity of staining to arrive at an index number that is predictive of endocrine response.

The fact that ER by IHC can be performed on minute quantities of tissue is a distinct advantage, especially for patients with a diagnosis of carcinoma by FNA.

Rosen was the first to describe tumor histopathologic correlations with ER expression. Tumor types that tend to be ER+ include tubular, mucinous, and papillary carcinomas, along with ductal carcinomas of low nuclear grade.

MATERIALS AND METHODS

Government Rajaji Hospital is a tertiary care centre in Madurai, Tamilnadu. It has the privilege of having maximum number of outpatients in South Tamilnadu.

Carcinoma breast is one of the common surgical problems presenting to our everyday outpatient department. Modified radical mastectomy is a common surgical procedure being performed in our operation theatres.

The purpose of this study is to compare the "Estrogen Receptor Status" between premenopausal and post menopausal women with carcinoma breast .

Duration of the study : from September 2010 to August 2012 .

SELECTION OF STUDY SUBJECTS

Genders eligible for study	:	Females only .
Age eligible for study	:	
Premenopausal	:	30-40 years
Postmenopausal	:	> 50 years

INCLUSION CRITERIA :

1. All patients with carcinoma breast attending the out patient department of surgery at Government Rajaji Hospital, Madurai are included .The clinical finding should be in favour of carcinoma breast .The malignancy should be proved either by FNAC or Trucut biopsy – HPE report .

2. The patient should have a clearcut menstrual history with regular menstrual periods .

EXCLUSION CRITERIA :

1. Patients with intake of exogenous estrogen exposure in the form of "Hormone Replacement Therapy" or prolonged intake of OCP .
2. Patients who had undergone hysterectomy previously ie. Surgical menopause.

DATA COLLECTION :

1. Menstrual History in detail

Premenopausal

- Age at menarche
- Menstrual cycles regular / irregular
- Length of each cycle
- LMP

Postmenopausal

- Age at menarche
- Age at menopause
- Last menstrual cycle

2. Marital status
3. Parity
4. Age of first child birth
5. Breast feeding history

- whether each child was breastfed or not ?

- if breastfed / for how long ?

- whether both breasts were equally used ?

6. Drug history :

   H/O prolonged OCP intake

   H/O hormone replacement therapy

7. Family history

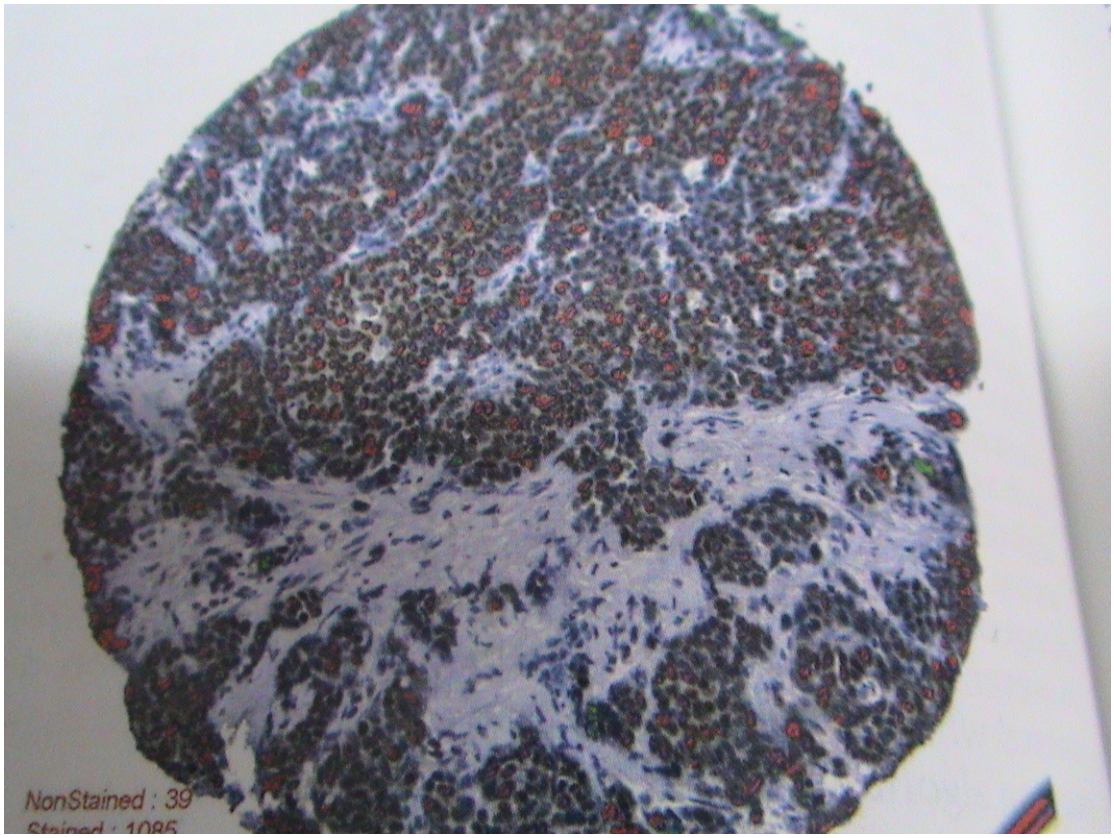
8. Clinical finding in favour of CA Breast

9. Investigation in favour of CA Breast

   - FNAC report

   - Trucut biopsy HPE report

ER STAINING



Reference Range

Quick Score = 0-8

Proportion

0 = No nuclear staining

1 = < 1% nuclei staining

2 = 1-10 % nuclei staining

3 = 11-33 % nuclei staining

4 = 34-66% nuclei staning

5 = 67-100% nuclei staining

INTENSITY

0 = No staining

1 = Weak staining

2 = Moderate staining

3 = Strong staining

INTERPRETATION

Patients with tumors scoring 2 or less are regarded as ER negative and have a negligible chance of response to therapy .

RESULTS

Table -1

Age Distribution for Pre menopausal

PRE MENOPAUSAL			
Age	Positive	Negative	Total
30 - 35	0	4	4
36 - 40	16	5	21
Total	16	9	25
Mean age	36.84		
S.D	1.97		

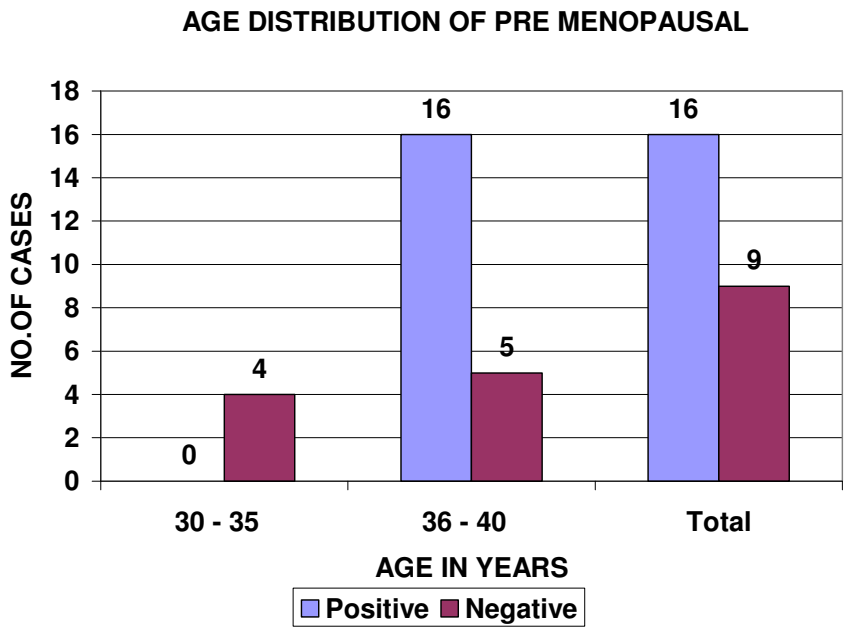

AGE DISTRIBUTION OF PRE MENOPAUSAL
NO.OF CASES
18
16
14
12
10
8
6
4
2
0
0
4
16
5
16
9
30 - 35
36 - 40
Total
AGE IN YEARS
Positive
Negative

Table -2

Age Distribution for Post menopausal

POST MENOPAUSAL			
Age	Positive	Negative	Total
50 - 60	15	4	19
61 - 70	4	0	4
71 - 80	2	0	2
Total	21	4	25
Mean	59.0		
S.D	7.86		
P value	< 0.001		

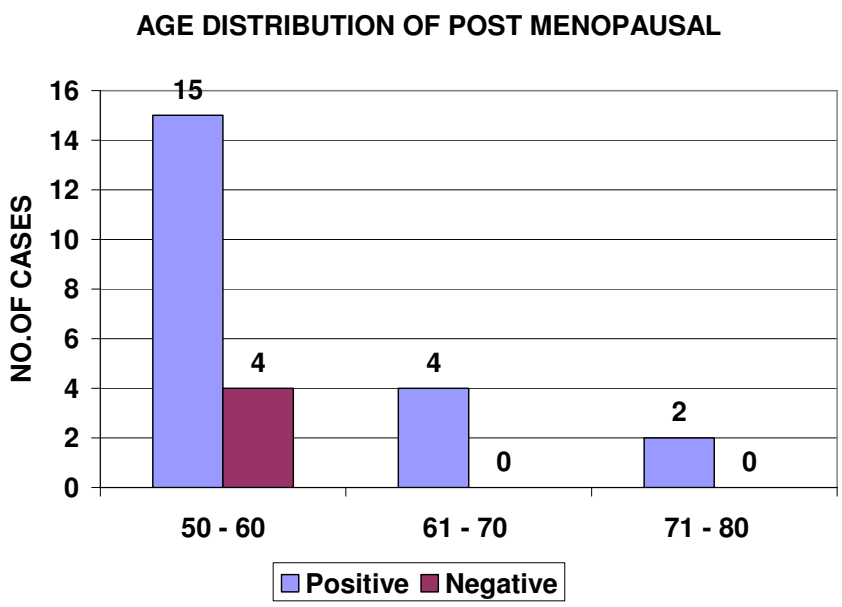

AGE DISTRIBUTION OF POST MENOPAUSAL
NO.OF CASES
16
14
12
10
8
6
4
2
0
15
4
4
0
2
0
50 - 60
61 - 70
71 - 80
Positive
Negative

Table -3

Distribution of Positive and Negative

	Positive	Negative
Pre menopausal	16	9
Post menopausal	21	4

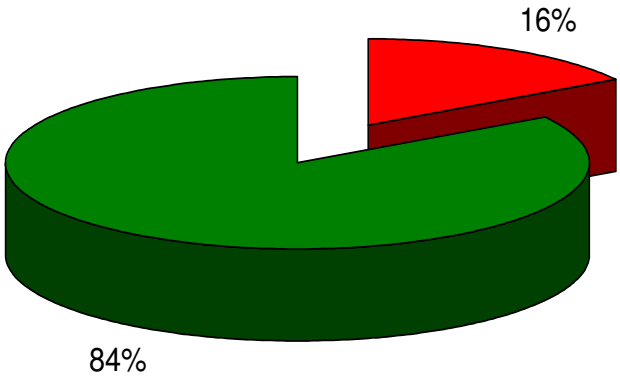
P value = 0.367 Not significant

(for Premenopausal)

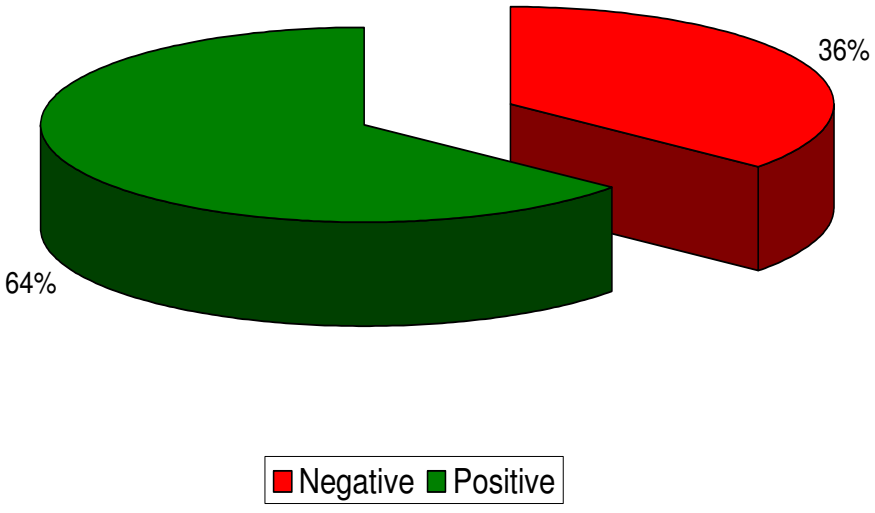
P value = 0.009 Significant (4 / 21)

(for Post menopausal)

POST MENOPAUSAL



PREMENOPAUSAL



DISCUSSION

Fifty patients with breast cancer included in this study, were divided into two subgroups. 25 premenopausal patients with age ranging from 30-40 and mean age 36.8 and post menopausal patients with age ranging from 50-80, with mean age 59.0 years.

Left breast was involved in 92 % of the cases and upper outer quadrant was the commonest site affected. Infiltrating ductal carcinoma was the underlying pathology in all the cases.

Estrogen receptor positivity was higher in postmenopausal patients (84%) than in premenopausal patients (64%). The difference is statistically significant.

The analyses of the data from patients with primary carcinoma of the breast indicate that CER increases with age from the third through the seventh decade. Most previous studies have emphasized menopausal status, suggesting that premenopausal patients have a lower incidence of CER positive tumors as well as quantitatively lower tumor levels of estrogen receptor compared with postmenopausal patients.

Martin et al found that although mean CER levels increased for each decade, significant difference were only noted between premenopausal and postmenopausal patients.

Similar study was conducted from Mansoura university from August 1986 to November 1987 , published in science and medical journal, vol.2 ,April 1990 . Estrogen receptor positivity was higher in postmenopausal patients than premenopausal patients. Hormonal treatment in estrogen receptor positive patients resulted in least number of failure of the treatment.

Patients of age group 45-65 years exhibited the higher frequency of estrogen receptor positivity than patients of age group 20-44 years. The difference was statistically significant ; this is in agreement with that previously reported by Pascual et al (1982) and Chua et al (1985)

ER positivity in postmenopausal females was higher than ER positivity in premenopausal females and this finding confirms the results obtained by Aboul Enein et al (1983).

This may be due to the elevated level of endogenous estrogen in plasma of premenopausal women which may mask receptor binding sites. (Hawkins et al . 1980 and knight et al. 1980).

CONCLUSION

In this study 50 post mastectomy specimens were collected (25-premenopausal and 25-postmenopausal) and subjected to estrogen receptor assay by biochemical monoclonal antibody method. Estrogen receptor status was correlated with age and menstrual status.

- The frequency of ER positivity increase steadily with age.
- The concentration and proportion of ER positivity were higher in postmenopausal than in premenopausal patients.
- ER assay is recommended as a routine test in the management of breast cancer because of its help in predicting the response to hormonal therapy, in addition its an important prognostic factor.

LIMITATIONS OF THE STUDY

1. Sample size should be more.

2. Technical problems in immunohistochemistry : like problems in fixation ; processing problems ; imprecise sectioning ; inappropriate staining.

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PROFORMA

Name : Age & Sex :

IP No. : Unit :

Occupation :

Address :

Date of Admission : Date of discharge :

Age at menarche : Age of menopause :

Menstrual history : Regular / Irregular

Length of cycle

LMP

Marital status : Parity :

Breast feeding Details :

Whether each child was breast fed or not

If breast fed for how long ?

Whether both breasts were equally used

Drug History : HRT / OCP

Family History :

Clinical finding in favour of CA breast

Investigation in favour of CA breast

FNAC report or

Trucut biopsy report

Investigation to be studied :

ER Status in post mastectomy specimen

MASTER CHART FOR POST MENOPAUSAL PATIENTS

S.No.	Name	Age	Side of CA Breast	IP No.	Marital Status	Parity	Breast feeding details	HRT	ER Status
1	Veerammal	70	left	73241	married	P3	breastfed adequately	Nil	positive
2	Krishnaveni	50	left	453219	married	P2	breastfed adequately	Nil	negative
3	Arayee	50	left	46789	married	P2	breastfed adequately	Nil	negative
4	Muthulakshmi	58	left	45759	married	P3	breastfed adequately	Nil	positive
5	Glori	80	left	74523	married	P4	breastfed adequately	Nil	positive
6	Arasammal	75	left	45326	married	P4	breastfed adequately	Nil	positive
7	Muniyammal	65	left	76894	married	P5	breastfed adequately	Nil	positive
8	Kaliammal	55	left	45423	married	P3	breastfed adequately	Nil	positive
9	Neela	60	left	48763	married	P2	breastfed adequately	Nil	positive
10	Lakshmi	60	left	45908	married	P2	breastfed adequately	Nil	positive
11	Dhanalakshmi	50	left	43984	married	P2	breastfed adequately	Nil	positive
12	Shabitha	55	left	75569	married	P3	breastfed adequately	Nil	positive
13	Sivagami	57	left	76142	married	P3	breastfed adequately	Nil	positive
14	Saraswathi	69	left	3897	married	P3	breastfed adequately	Nil	positive
15	Annammal	50	left	63786	married	P2	breastfed adequately	Nil	positive
16	Rajeshwari	55	left	23678	married	P2	breastfed adequately	Nil	positive
17	Mariyambeevi	60	left	59987	married	P3	breastfed adequately	Nil	positive
18	Ramayee	60	left	56732	married	P3	breastfed adequately	Nil	positive
19	Panchavarnam	51	right	87543	married	P2	breastfed adequately	Nil	positive

20	Mariyammal	56	right	58762	married	P2	breastfed adequately	Nil	positive
21	Rajammal	51	right	98712	married	P3	breastfed adequately	Nil	negative
22	Avudaiyammal	60	left	90078	married	P3	breastfed adequately	Nil	positive
23	Janakiammal	62	right	50065	married	P4	breastfed adequately	Nil	positive
24	Thangam	53	left	76985	married	P2	breastfed adequately	Nil	negative
25	Rani	58	left	63489	married	P2	breastfed adequately	Nil	positive

MASTER CHART FOR PRE MENOPAUSAL PATIENTS

S.No.	Name	Age	Side of CA Breast	IP No.	Marital Status	Parity	Breast feeding details	HRT	ER Status
1	Gandhimathi	39	left	93419	married	P2	breastfed adequately	Nil	positive
2	Nanjammal	39	left	68768	married	P3	breastfed adequately	Nil	positive
3	Shenbagavalli	38	left	58802	married	P3	breastfed adequately	Nil	positive
4	Kalyani	37	left	85107	married	P2	breastfed adequately	Nil	positive
5	Amaravathi	36	left	34214	married	P2	breastfed adequately	Nil	positive
6	Gowri	38	left	36432	married	P1	breastfed adequately	Nil	positive
7	Shantha	36	left	98765	married	P1	breastfed adequately	Nil	positive
8	Janaki	35	left	13567	married	P2	breastfed adequately	Nil	negative
9	Nirmalanavamani	38	left	65438	married	P3	breastfed adequately	Nil	positive
10	Latha	38	left	65457	married	P2	breastfed adequately	Nil	positive
11	Pounthai	30	left	96473	married	P2	breastfed adequately	Nil	negative
12	Muthuselvi	36	left	10362	married	P2	breastfed adequately	Nil	positive
13	Chithra	38	left	8921	married	P3	breastfed adequately	Nil	positive
14	Packiam	34	left	41321	married	P2	breastfed adequately	Nil	negative
15	Kamatchi	37	left	34865	married	P1	breastfed adequately	Nil	positive
16	Murugeswari	37	left	80178	married	P2	breastfed adequately	Nil	negative
17	Meenakshi	36	left	83969	married	P2	breastfed adequately	Nil	negative
18	Selvi	37	left	88653	married	P3	breastfed adequately	Nil	negative
19	Nisha	38	left	77632	married	P3	breastfed adequately	Nil	positive
20	Sundari	39	left	73902	married	P2	breastfed adequately	Nil	positive
21	Priya	38	left	21576	married	P2	breastfed adequately	Nil	positive
22	Bhanu	36	right	26207	married	P2	breastfed adequately	Nil	negative

23	Vellayammal	39	right	62564	married	P3	breastfed adequately	Nil	positive
24	Nagammal	35	right	57080	married	P2	breastfed adequately	Nil	negative
25	Saraswathi	37	right	73591	married	P2	breastfed adequately	Nil	negative

Ref. No. 00216 /E4/3/2011 Govt.Rajaji Hospital,Madurai.20.
Dated: .01.2012

Institutional Review Board / Independent Ethics Committee.
Dr. A. Edwin Joe, M.D (FM), BL.,
Dean, Madurai Medical College & 2521021 (Secy)
Govt Rajaji Hospital, Madurai 625020.
Convenor
grhethicssecy @gmail.com.

Sub: Establishment-Govt. Rajaji Hospital, aMadurai-20-
Ethics committee-Meeting Agenda-communicated-regarding

The next Ethics Committee meeting of the Govt. Rajaji Hospital, Madurai was held at 11.00 Am to 1.00Pm on 27.01.2012 at the Dean Chamber, Govt. Rajaji Hospital, Madurai. The following members of the committee have been attended the meeting.

1. Dr.N.Vijayasankaran,M.ch(Uro.) 094-430-58793 0452-2584397	Sr.Consultant Urologist Madurai Kidney Centre, Sivagangai Road,Madurai	Chairman
2. Dr.P.K. Muthu Kumarasamy, M.D., 9843050911	Professor & H.O.D of Medical, Oncology(Retired)	Member Secretary
3. Dr.T.Meena,MD 094-437-74875	Professor of Physiology, Madurai Medical College	Member
4. Dr. S. Thamilarasi, M.D (Pharmacol)	Professor of pharmacology	
5.Dr.Moses K.Daniel MD(Gen.Medicine) 098-421-56066	Professor of Medicine Madurai Medical College	Member
6.Dr.M.Gobinath,MS(Gen.Surgery) 097-871-50040	Professor of Surgery Madurai Medical College	Member
7.Dr.S. Dilshadh, MD(O&G)	Professor of OP&Gyn Madurai Medical College	Member
8.Dr.S.Vadivel Murugan., M.D, 097-871-50040	Professor of Medicine Madurai Medical College	Member
9.Shri.M.Sridher,B.sc.B.L. 099-949-07400	Advocate, 623-B.II.Floor,East II Cross, K.K.Nagar,Madurai.20.	Member
10.Shri.O.B.D.Bharat,B.sc., 094-437-14162	Businessman Plot No.588, K.K.Nagar,Madurai.20.	Member
11.Shri. S.sivakumar,M.A(Social) Mphil 093-444-84990	Sociologist, Plot No.51 F.F, K.K Nagar, Madurai.	Member

Following Projects were approved by the committee

Sl. No	Name of P.G	Course	Name of the Project	Remarks
1.	V.Gopisri	PG. M.S (genl Surg)	Breast cancer, hormone receptor status and response; a clinical study.	Approved

Please note that the investigator should adhere the following; She/He should get a detailed informed consent from the patients/participants and maintain Confidentially.

1. She/He should carry out the work without detrimental to regular activities as well as without extra expenditure to the institution to Government.
2. She/He should inform the institution Ethical Committee in case of any change of study procedure site and investigation or guide.
3. She/He should not deviate for the area of the work for which applied for Ethical clearance.
She/He should inform the IEC immediately, in case of any adverse events pr Serious adverse reactions.
4. She/he should abide to the rules and regulations of the institution.
5. She/He should complete the work within the specific period and apply for if any Extension of time is required She should apply for permission again and do the work.
6. She/He should submit the summary of the work to the Ethical Committee on Completion of the work.
7. She/He should not claim any funds from the institution while doing the word or on completion.
8.She/He should understand that the members of IEC have the right to monitor the work with prior intimation.

DEAN

To
All the above members and Head of the Departments concerned.
All the Applicants.

Forwarded

Professor and Head
Department of Surgery
MADURAI MEDICAL COLLEGE
Govt. Rajaji Hospital
Madurai-20

PLAGIARISM CERTIFICATE